A New *Metaculus* S[illegible]s (Acari: Eriophyoidea) on *Diplotaxis tenuifolia* (Brassicac[illegible]rom Serbia: A Combined Description Using Mor[illegible]gy and DNA Barcode Data

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ABSTRACT A new species of e[illegible] mite, *Metaculus diplotaxi* n. sp. inhabiting *Diplotaxis tenuifolia* (L.) DC., is described from S[illegible] investigate interspecific variability between *Metaculus* spp. on three different Brassicaceae host plants (viz. *D. tenuifolia, Lepidium latifolium* (L.), *Isatis tinctoria* (L.)), we analyzed phenotypic variability of morphological traits and molecular sequences of the mitochondrial cytochrome oxidase subunit I (MT-CO1). Discriminant analysis identified seven traits that significantly differentiate three *Metaculus* spp: *M. lepidifolii, M. rapistri*, and *M. diplotaxi* n. sp. Analysis of MT-CO1 sequences supported the results obtained from the analysis of morphometric features.

KEY WORDS weed biological control, Eriophyidae, mite, morphological trait, MT-CO1

To date, 12 species of eriophyoid mites belonging to the genus *Metaculus* Keifer have been described worldwide. One of them, *Metaculus mangiferae* (Attiah), is considered to be an economically important pest of mango; eight additional species were described associated with woody plants in India and Africa, while another, *M. tanythrix* Flechtman and Queiroz, was found on a tree fern in Brasil (Attiah 1955; Keifer 1962; Mohanasundaram 1980, 1982, 1983; Meyer (Smith) 1990; Amrine et al. 2003; Flechtmann and De Queiroz 2010). Only two of them were recorded on plants of the family Brassicaceae: *M. rapistri* Carmona on *Rapistrum rugosum* (L.) All and *Isatis tinctoria* L.; and *M. lepidifolii* Monfreda and De Lillo on *Lepidum latifolium* L. (Carmona 1969, Monfreda and De Lillo 2012).

Metaculus rapistri was initially described by Carmona (1969) from samples of *R. rugosum* collected in Portugal. Thereafter, a supplementary morphological description of this species was provided by Monfreda and De Lillo (2012) from samples of *Isatis tinctoria* collected in Turkey. During our investigations of eriophyoids in Serbia, another mite of the genus *Metaculus* was recorded on *Diplotaxis tenuifolia* (L.) DC. The Brassicaceae is a large family of about 338 genera and >3,700 species distributed worldwide (Warwick et al. 2006). The genus *Diplotaxis* is native to the Mediterranean region and comprises about 30 species (Warwick et al. 2006). Of these, only two species, *Diplotaxis tenuifolia* (L.) DC. and *Diplotaxis muralis* (L.) DC., have been recorded in the Flora of Serbia (Jovanović-Dunjić 1975).

Due to minute size and simple body construction, there are few structural characteristics useful in the systematics of eriophyoid mites compared to those of most other mite taxa (Lindquist and Amrine 1996); therefore, the authors decided to use an integrated systematics approach to analysis of these *Metaculus* spp., based on the combination of morphological and genetic approaches. To date, several studies assessing intraspecific variation, host-adapted strains, and cryptic species of Eriophyoidea have been published (Skoracka et al. 2002, 2012, 2013, 2014; Navia et al. 2006; Magud et al. 2007; Vidović et al. 2010, 2014; Miller et al. 2013; Lewandowski et al. 2014; Li et al. 2014). To supplement classical descriptive methods, DNA barcoding, using predominantly the nucleotide sequence of the mitochondrial cytochrome oxidase gene subunit I (MT-CO1), has been utilized as an effective method to distinguish between animal species (e.g., Hebert et al. 2003, Navajas and Navia 2010, Gaskin et al. 2011).

In the past few decades, increasing interest in the use of eriophyoid mites as biological control agents has been expressed, especially due to their high host-specificity, high intrinsic rate of reproduction and damage frequently caused to reproductive plant parts (Andres 1983, Rosen and Huffaker 1983, Briese and Cullen 2001, Skoracka et al. 2010, Smith et al. 2010).

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Among a significant number of arthropods selected, investigated and consequently released as potential biological control agents, some are eriophyoid mites, including in the genus *Metaculus* (Smith et al. 2010). Given that the plant species *Isatis tinctorium* and *Lepidium latifolium* have been selected as target weeds for classical biological control programs in the United States (B. Rector, personal communication), it is necessary to know the precise taxonomic status of these mites, as well as their ecological characteristics, especially their host-plant relationships.

The goal of this study was to clarify the taxonomic status of mite species within the genus *Metaculus* that inhabit host plants of the family Brassicaceae. This paper presents results of quantitative morphological analyses, as well as MT-CO1 nucleotide sequence comparisons for three *Metaculus* populations inhabiting three different cruciferous host plants. The resulting description of a new eriophyoid mite species, *Metaculus diplotaxi* n. sp., is also presented.

Materials and Methods

Collection and Morphological Measurements. Plant samples of *D. tenuifolia* were collected in Zemun, Serbia (44° 49.238′ N; 20° 21.372′ E); *L. latifolium* was collected in Rustavi, Georgia (41° 32.917′ N; 44° 59.799′ E); and *I. tinctoria* in Goreme, Turkey (38° 38.24′ N; 34° 51.105′ E). Mites were extracted from the plant material using methods described by De Lillo (2001). The specimens from each sample were mounted in Keifer's F medium (Keifer 1975). Mites were studied under a phase-contrast microscope (LEICA DMLS, Leica, Wetzlar, Germany). Morphometry was performed using the software package IM 1000 (Leica, Wetzlar, Germany).

For the species description of *Metaculus diplotaxi* n. sp., measurements are given in micrometers (μm) and, unless stated otherwise, refer to the length of the structure. Each measurement of the holotype precedes the corresponding range for paratypes (given in parentheses). Terminology of eriophyoid morphology and nomenclature follow Lindquist (1996) and systematic classification is based on Amrine et al. (2003). Measurements and illustrations were made according to Amrine and Manson (1996) and De Lillo et al. (2010).

For the morphometric study, 28–30 females from each sample were examined in the dorso-ventral position. Twenty-two morphological traits of each individual were measured (see measured traits in Table 1). The data were tested for normality using Kolmogorov–Smirnov tests. All analyzed variables were distributed normally, with homogeneity of variance. A one-way multivariate analysis of variance (MANOVA) was used to examine the differences in morphological variation among the three groups of mites. Canonical discriminant analysis (CDA) was performed to visualize differences among three groups and to determine the relative importance of characters as discriminators among groups. All statistical analyses were conducted using the Statistica 6 software package (StatSoft 2001).

All material examined is deposited in the Acarology Collection, Department of Entomology and Agricultural Zoology, Faculty of Agriculture, University of Belgrade, Serbia.

Scanning Electron Microscopy. Scanning electron micrographs (SEM) were made according to Alberti and Nuzzaci (1996) using a scanning electron microscope (JEOL-JSM 6390, JEOL GmbH, Munich, Germany) at the Faculty of Agriculture, University of Belgrade. Live mites were collected individually with a fine entomological needle from fresh plant parts under a stereomicroscope and placed in the SEM sample holder.

Table 1. Basic statistical data for 22 morphological traits of three species from different host plants

Traits	*M. diplotaxi* n. sp. ($n = 30$)		*M. lepidifolii* ($n = 30$)		*M. rapistri* ($n = 28$)	
	Mean	SD	Mean	SD	Mean	SD
A: body length	198.61	7.42	261.27	11.88	228.37	15.15
B: prodorsal shield length	32.01	3.33	35.86	1.90	37.73	2.44
C: prodorsal shield width	53.64	4.18	53.16	3.39	60.66	4.78
D: setae *sc* length	19.60	2.01	28.74	1.70	27.20	2.53
E: tubercles *sc* apart	29.33	3.07	27.78	1.25	31.21	1.86
F: number.of dorsal annuli	33.50	1.57	46.53	2.05	38.14	3.60
G: number of ventral annuli	66.00	1.26	60.97	3.49	66.21	4.21
H: setae *c2* length	31.04	3.20	50.91	3.92	33.92	1.84
I: setae *d* length	42.94	9.34	64.31	3.74	62.15	4.30
J: setae *e* length	11.45	1.90	19.92	1.79	14.76	1.81
K: setae *f* length	26.10	2.93	33.28	2.47	28.37	2.14
L: genitalia length	15.17	2.02	13.99	0.60	14.39	0.95
M: genitalia width	22.51	1.76	24.40	1.02	24.65	1.23
N: setae *3a* length	13.64	1.39	19.28	1.77	15.68	1.15
O: tubercles *3a* apart	14.93	1.17	18.80	1.07	17.19	1.42
R: tubercles *1a* apart	9.32	1.56	8.43	0.66	9.96	0.80
S: tubercles *2a* apart	21.34	1.55	24.54	1.36	25.41	1.80
T: setae *2a* length	31.77	4.99	43.10	2.62	41.78	4.77
U: tibia I length	8.60	0.99	7.99	0.45	8.56	0.56
W: tarsus I length	4.88	1.14	6.16	0.44	6.40	0.48
V: tibia II length	7.18	0.70	7.13	0.45	8.01	0.65
Z: tarsus II length	5.69	0.86	5.87	0.37	6.14	0.42

Table 2. Characteristics of the specimens used in this study

	Mite species	Host plant	Locality–date	Accession numbers COI
1	*Metaculus lepidifolii*	*Lepidium latifolium*	Turkey-Incesu–June 2012 (38° 37.20′ N, 35° 11.05′ E).	KP861853
2	*Metaculus lepidifolii*	*Lepidium latifolium*	Georgia-Rustavi–May 2014 (41° 32.917′ N, 44° 59.799′ E)	KP861854
3	*Metaculus rapistri*	*Isatis tinctoria*	Turkey-Goreme–March 2013 (38° 38.24′ N, 34° 51.105′ E)	KP861855
4	*Metaculus rapistri*	*Isatis tinctoria*	Turkey-Goreme–April 2014 (38° 38.24′ N, 34° 51.105′ E)	KP861856
5	*Metaculus diplotaxi* n. sp.	*Diplotaxis tenuifolia*	Serbia-Zemun–September 2010 (44° 49.238′ N, 20° 21.372′ E)	KP861857
6	*Metaculus diplotaxi* n. sp.	*Diplotaxis tenuifolia*	Serbia-Zemun–June 2012 (44° 49.238′ N, 20° 21.372′ E)	KP861858

CLSM Technique. Confocal laser scanning microscopy (CLSM) acquisition was carried out in the Center of Microscopy and Microanalysis of Saint-Petersburg State University (Russia) using a spectral confocal and multiphoton system (Leica TCS SP2, Leica, Wetzlar, Germany) with objectives of 63x N.A. (1.4–0.60 Oil lBL HCX PL APO) and 40x N.A. (1.25–0.75 Oil CS HCX PL APO) at an excitation wavelength of 405 nm (blue laser) with the same adjustments of the confocal microscope as described by Chetverikov et al. (2014) and Asadi et al. (2014). Image stacks were rendered three dimensionally with the aid of the reconstruction software Amira5.3.2 (FEI Visualization Science Group, Hillsboro, OR).

DNA Extraction, PCR Amplification, and Sequencing. Material subject to molecular analysis included populations of mites collected in Georgia, Serbia, and Turkey. Sampling localities and data related to the host plants are summarized in Table 2. Mite specimens for molecular analysis were preserved in 96% ethanol and stored at 4°C until DNA extraction. Total DNA was extracted from 30 to 40 whole specimens using QIAGEN DNeasy Blood & Tissue Kit (QIAGEN, Hilden, Germany), according to the manufacturer's instructions, with modifications based on Dabert et al. (2008). The MT-CO1 sequence was amplified using the pair of primers LCO1490/HCO2198 (Folmer et al. 1994). Polymerase chain reactions (PCR) were conducted using High Yield Reaction Buffer A with Mg (1x), 2.5 mM $MgCl_2$, 0.6 mM of each dNTP, 0.5 µM of each primer, and 1 U of KAPA*Taq* DNA polymerase (Kapa Biosystems, London, United Kingdom) in a 25 µl final volume. PCR was carried out in a Mastercycler ep gradient S thermal cycler (Eppendorf, Hamburg, Germany) applying the following steps: 95°C for 5 min (initial denaturation), 35 cycles at 94°C for 1 min, 1 min at 54°C (annealing), 1 min 30 s at 72°C, with a final extension at 72°C for 7 min. PCR amplicons were purified using the QIAquick PCR purification Kit (QIAGEN, Hilden, Germany) according to the manufacturer's instructions, and sequenced on automated equipment by Macrogen (Seoul, South Korea) with the same primer pairs as in the initial PCR procedure. Sequences are available from GenBank under accession numbers (KP861853–KP861858). The sequences were manually edited using FinchTV v.1.4.0 (www.geospiza.com), and aligned by CLUSTAL W integrated in MEGA5 software (Tamura et al. 2011). Uncorrected pairwise genetic distances were used to calculate the average genetic distance between species with different host affiliations.

Nomenclature. This paper and the nomenclatural act it contains have been registered in Zoobank (www.zoobank.com), the official register of the International Commission on Zoological Nomenclature. The LSID (Life Science Identifier) number of the publication is: urn:lsid:zoobank.org:pub:79789579-F377-4B59-8B42-B5E757E01984.

Results

Morphometrical Analyses. Descriptive statistics of *Metaculus* spp. quantitative traits are given in Table 1. The one-way MANOVA of the three *Metaculus* spp. populations revealed significant differences in the morphological variation of 22 commonly studied morphological traits (Wilks' Lambda = 0.002043 (44, 128) = 31.452; $P < 0.0001$).

The results of the discriminate analysis of 22 traits showed distinct discrimination between populations of *M. lepidifolii* from *L. latifolium* and its two congeners, *M. rapistri* from *I. tinctoria* and *M. diplotaxi* n. sp. from *D. tenuifolia*, based on the first canonical axis (Fig. 1). Discrimination was also shown between *M. rapistri* from *I. tinctoria* and the two other *Metaculus* populations based on the second canonical axis (Fig. 1). The first canonical function described 88.2% of the total variability, while the second canonical function described 11.8% (Fig. 1). The number of dorsal and ventral annuli and the lengths of the lateral setae (*c2*), the second ventral setae (*e*), and the first tibia were the traits with the most discriminative power based on the first canonical function. This function clearly separated *M. lepidifolii* collected from *L. latifolium* from the other two *Metaculus* populations (see Table 3).

Molecular Analyses. The final alignment of the MT-CO1 sequences consisted of 585 bp. No insertions or deletions were found between the MT-CO1 sequences. In total, 160 (27.4%) nucleotides were polymorphic, of which 157 were parsimony informative. The translation of the nucleotide sequence resulted in a 195 amino acid positions, of which 19 (9.7%) were variable. The average mean divergence over all the sequence pairs was 15.2% and ranged from 0 to 22.6% (Table 4, Fig. 1). *Metaculus lepidifolii* populations collected from *L. latifolium* in two localities—Turkey and Georgia—revealed the existence of two haplotypes with a genetic divergence of 0.7%. No intraspecific nucleotide sequence divergence was recorded for populations of *M. rapistri* collected on *I. tinctoria* or for the populations of *M. diplotaxi* n. sp. collected on *D. tenuifolia*.

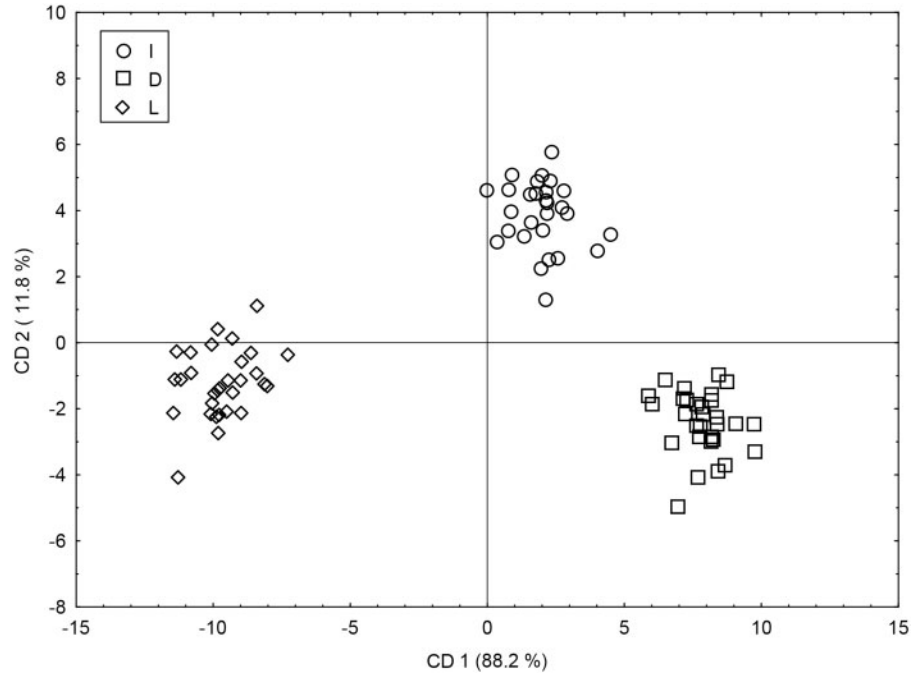

Fig. 1. Plot of scores of the first two canonical axes (CD1 and CD2) of three populations of *Metaculus* spp. scored for 22 commonly studied morphological traits; I = mites from *Isatis tinctoria*, D = mites from *Diplotaxis tenuifolia*; L = mites from *Lepidium latifolium*.

Table 3. Standardized coefficients for canonical variables on two canonical axes in discriminant function analysis on 22 commonly studied morphological traits in eriophyoid mite studies

Traits	CD1	CD2
A- body length	−0.173	0.056
B- prodorsal shield length	−0.002	0.190
C- prodorsal shield width	0.178	0.115
D- setae *sc* length	−0.188	0.794
E- tubercles *sc* apart	0.271	0.145
F- no. of dorsal annuli	−1.190	−0.122
G- no. of ventral annuli	0.855	0.431
H- setae *c2* length	−0.516	−0.566
I- setae *d* length	−0.175	0.541
J- setae *e* length	−0.497	−0.131
K- setae *f* length	−0.031	−0.194
L-genitalia length	−0.021	−0.038
M- genitalia width	0.044	0.053
N- setae *3a* length	−0.168	−0.150
O- tubercles *3a* apart	−0.079	−0.021
R- tubercles *1a* apart	0.045	−0.065
S- tubercles *2a* apart	−0.098	0.504
T- setae *2a* length	−0.113	0.110
U- tibia I length	0.412	−0.286
W- tarsus I length	−0.126	0.318
V- tibia II length	−0.178	0.185
Z- tarsus II length	0.083	−0.323
Eigenvalues	56.214	7.555
Cumulative proportions	0.882	1.000

Bold text indicates the traits that had the most discriminative power.

Taxonomy

(Figs. 2 and 3)

Metaculus diplotaxi n. sp. Petanović et Vidović 2015

(urn:lsid:zoobank.org:act:B8263A90-8670-438A-92B8-EA15D3F86675)

Description

Female. ($n = 10$). Body wormlike 190 (182–211), 71 (63–76) wide, whitish in color. Gnathosoma 19 (18–20) cured downwards, chelicerae 11 (11–15), setae *d* 4 (3–5). Prodorsal shield 32 (31–38), 62 (52–62) wide, triangular with pointed frontal lobe over gnathosoma. Prodorsal shield pattern almost obscure, without median line but with two slightly curved admedian lines on rear 3/4 of shield and two submedian lines almost parallel to the lateral margin of shield, anteriorly connecting with the admedian lines. The anterior part of the admedian lines are curved convexly whereas the posterior ends of the admedian lines are concave. On some specimens one more line was observed between the admedian lines, near the posterior margin of the shield. Tubercles *sc* are on rear shield margin 31 (25–34) apart, scapular setae *sc* 18 (17–23). **Leg I** 33 (32–36); femur 10 (8–11), setae *bv* 11 (10–12); genu 6 (4–6), setae *l″* 21 (19–24); tibia 7 (7–9), setae *l′* 3 (3–5); tarsus 6 (5–7), setae *ft′* 16 (13–17), setae *ft″* 21 (18–22); tarsal solenidion *ω* 6 (5–8), distally slightly rounded; tarsal empodium 4 (3–6), 4-rayed. **Leg II** 28 (25–31); femur 8 (7–10), setae *bv* 9 (6–9); genu 6 (5–7), setae *l″* 5 (4–7); tibia 6 (6–8); tarsus 7 (5–7), setae *ft′* 4 (4–5), setae *ft″* 16 (15–20); tarsal solenidion *ω* 6 (5–7), distally

Table 4. Uncorrected p-distances among *Metaculus* spp. populations, grouped according to their origin

	Population	1	2	3	4	5
1	*Metaculus lepidifolii* ex. *Lepidium latifolium*					
2	*Metaculus lepidifolii* ex. *Lepidium latifolium*	0.007				
3	*Metaculus rapistri* ex. *Isatis tinctoria*	0.219	0.221			
4	*Metaculus rapistri* ex. *Isatis tinctoria*	0.219	0.221	0.000		
5	*Metaculus diplotaxi* n. sp. ex. *Diplotaxis tenuifolia*	0.226	0.226	0.121	0.121	
6	*Metaculus diplotaxi* n. sp. ex. *Diplotaxis tenuifolia*	0.226	0.226	0.121	0.121	0.000

Population numbers refer to populations collected from locations as listed in Table 2.

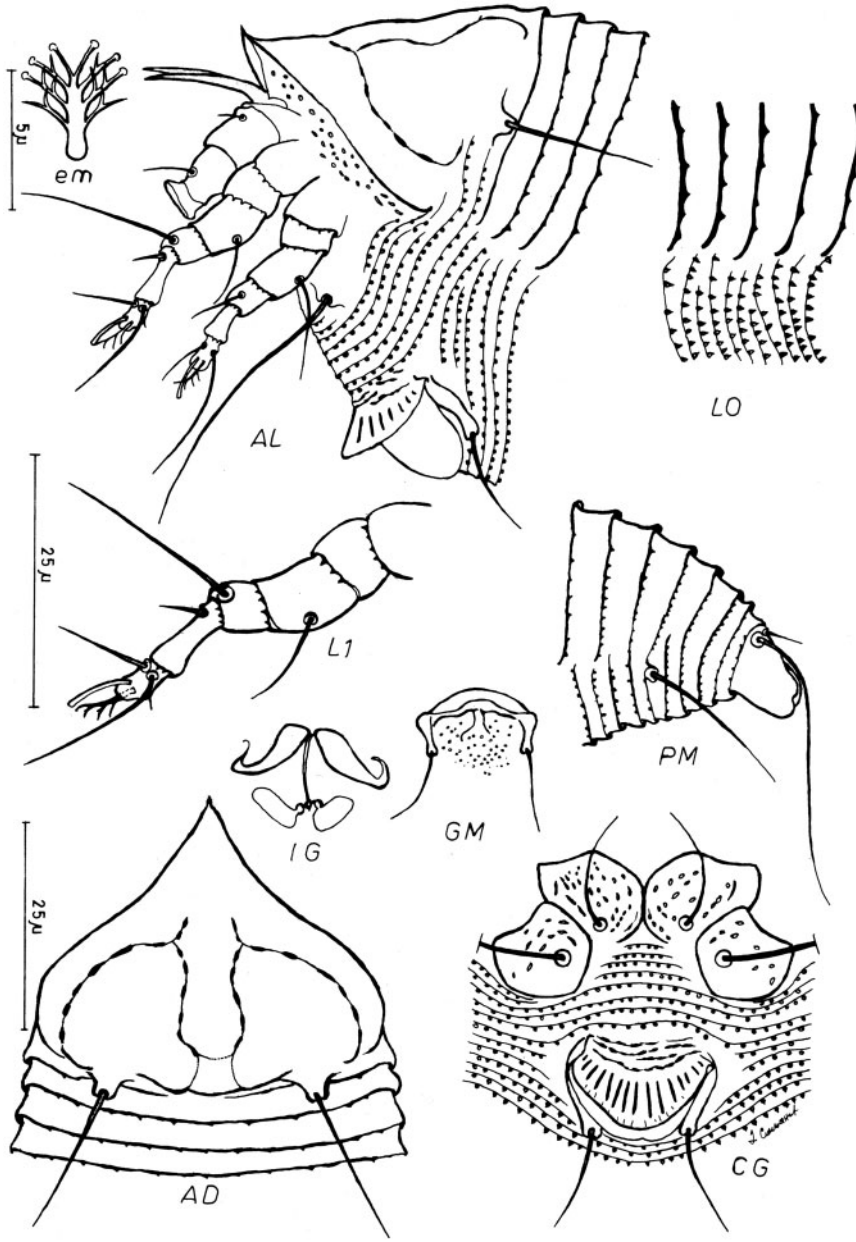

Fig. 2. Semischematic drawings of *Metaculus diplotaxi* n. sp. (female unless specified otherwise). Abbreviations: AD, antero-dorsal body; AL, antero-lateral body; CG, coxigenital region; em, empodium; GM, genital region, male; IG, internal genitalia, female; L1, Leg I; LO, lateral opisthosoma; PM, postero-lateral body (telosoma).

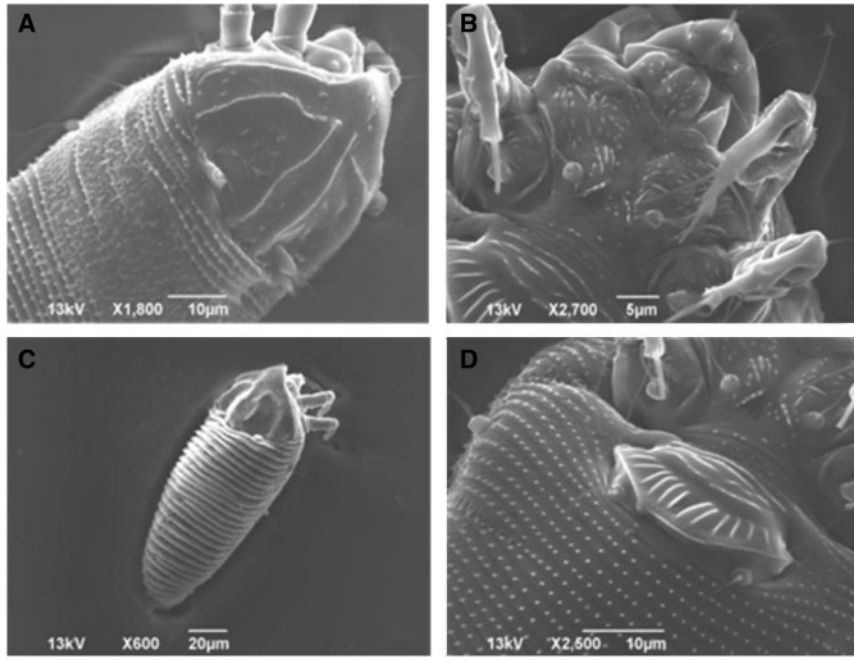

Fig. 3. SEM images of *Metaculus diplotaxi* n. sp. (A) prodorsal shield, (B) coxigenital region, (C) dorsal view, (D) genital region.

rounded; tarsal empodium 4 (4–6), 4-rayed. **Coxae** partially with short dashes; setae *1b* absent, setae *1a* 18 (17–18), *1a* tubercles 10 (8–11) apart, seta *2a* 30 (26–43), *2a* tubercles 22 (21–24) apart. Sternal line 7 (6–9) long. **Genitalia coverflap** 17 (13–17), 23 (23–25) wide, with 11 (10–13) longitudinal striae in single row; setae *3a* 12 (11–15), 15 (13–17) apart. Opisthosoma with subequal annuli: 32 (29–35) dorsal and 66 (64–70) ventral annuli. Dorsal opisthosoma with pointed microtubercles on rear margins of annuli; ventral microtubercles pearl like. Setae *c2* 33 (28–35), 66 (56–66) apart, on annulus 11 (9–12); setae *d* 38 (33–57), 44 (33–44) apart, on annulus 25 (25–27); setae *e* 11 (9–15), 23 (17–23) apart, on annulus 46 (39–46); setae *f* 23 (22–29), 22 (21–23) apart, on annulus 60 (56–66); seta *h2* 50 (27–54), 10 (8–10) apart; setae *h1* 2 (2–4), 6 (5–6) apart.

Male. ($n = 3$). Similar to female. Prodorsal shield 26 (26–33), 48 (44–54); opisthosoma with 30–32 dorsal semiannuli and 60–61 ventral semiannuli; setae *d* 49 (39–59); setae *2a* 24 (22–25).

Type Material. HOLOTYPE: 1 female, SERBIA: Zemun (44° 49.23.88′ N; 20° 21.37.28′ E), 20-IX-2010, on *Diplotaxis tenuifolia*, D. Smiljanić. PARATYPES: 50 females, 6 males, same data.

Etymology. The species designation is from the specific name of the host plant, masculine noun in genitive case.

Host Plant. *Diplotaxis tenuifolia* (L.) DC. (Brassicaceae), commonly known as "perennial wall rocket" or "wild rocket" or "sand rocket" or "Lincoln weed" (see Fig. 4).

Differential Diagnosis and Remarks

Metaculus diplotaxi n. sp. is close to *M. rapistri*, but differences in some morphometric characters can be noticed. For example, *M. diplotaxi* n. sp. has generally a larger and wider body with a wider prodorsal shield, higher number of ventral annuli, fewer dorsal annuli and longer *c2* setae but shorter *sc*, *d* and *2a* setae (Table 5). The shield pattern of both species is almost obscure, but the shapes of their respective admedian lines differs notably, making this the most important qualitative trait by which these species can be recognized. The anterior part of the admedian lines are curved convexly in *M. diplotaxi* n. sp., while in *M. rapistri* they are concave. The admedian lines in *M. diplotaxi* n. sp. are also more straight and closer together than in *M. rapistri* (Fig. 5A–C). In addition, in *M. diplotaxi* n. sp. the short lines anterior to the epigynium are broken, while in *M. rapistri* they are parallel to each other and unbroken (Fig. 5D and E).

The new species, *M. diplotaxi* n. sp., differs from *M. lepidifolii* mainly by the prodorsal shield pattern. The shield pattern of *M. diplotaxi* n. sp. is almost obscure, unlike the *M. lepidifolii* whose shield pattern is composed of short lines which form cells in the median part of the shield. In addition to the shield pattern the two species can be distinguished by the following meristic characters: *M. diplotaxi* n. sp. has a higher number of ventral annuli and striae on the female coverflap; fewer dorsal annuli; and shorter *sc*, *2a*, *c2*, *d*, *e*, and *f* setae (Table 5).

Characteristics that had the highest impact on separation of these three species were the length of scapular (*sc*) setae, number of dorsal and ventral annuli, length of lateral (*c2*), first ventral (*d*), and second ventral (*e*) setae, distance between third coxal (*2a*) setae and length of first tibia (Table 3).

Fig. 4. *Diplotaxis tenuifolia* healthy (left) and plant with symptoms (right).

Table 5. Comparative measurements (with ranges in parentheses) of *Metaculus lepidifolii* from *Lepidium latifolium* (Monfreda and DeLillo 2012) collected in Turkey, *Metaculus rapistri* from *Isatis tinctoria* (Monfreda and De Lillo 2012) collected in Turkey, and *Metaculus diplotaxi* n. sp. from *Diplotaxis tenuifolia* collected in Serbia

Morphometric characters	*M. diplotaxi* n. sp Serbia ($n = 10$)	*M. lepidifolii* Turkey ($n = 10$)	*M. rapistri* Turkey ($n = 10$)
Length of body	190 (182–211)	200 (180–220)	158 (140–190)
Width of body	71 (63–76)	45 (40–50)	46 (41–58)
Length of setae *d*	4 (3–5)	8 (8–9)	6 (5–6)
Length of cheliceral stylets	11 (11–15)	21 (18–22)	16 (12–16)
Length of prodorsal shield	32 (31–39)	29 (27–32)	37 (30–37)
Width of prodorsal shield	62 (52–62)	35 (33–35)	47 (43–48)
Length of setae *sc*	18 (17–23)	28 (27–30)	26 (21–26)
Separation of tubercles *sc*	31 (25–34)	26 (23–27)	27 (25–29)
Length of leg I	33 (30–36)	37 (33–39)	37 (26–38)
Length of tibia I	8 (7–9)	8 (7–9)	9 (8–10)
Length of tarsus I	6 (5–7)	7 (6–7)	7 (6–8)
Length of solenidion ω I	6 (4–8)	8 (7–9)	8 (7–8)
Number of rays on empodium I	4	4	4
Length of leg II	28 (24–31)	32 (30–35)	35 (28–37)
Length of tibia II	6 (6–8)	7 (7–8)	8 (7–9)
Length of tarsus II	7 (5–8)	6 (6–7)	6 (6–7)
Length of solenidion ω II	6 (6–7)	8 (7–8)	7 (6–7)
Length of empodium II	4 (4–6)	5 (5–6)	4
Length of setae *1a*	18 (14–18)	17 (17–27)	15 (10–18)
Separation of tubercles *1a*	10 (8–11)	9 (9–10)	9 (7–10)
Length of setae *2a*	30 (26–44)	42 (38–47)	38 (23–42)
Separation of tubercles *2a*	22 (20–23)	23 (17–25)	23 (19–23)
Length of female genitalia	16 (10–17)	15 (14–17)	16 (13–17)
Width of female genitalia	23 (22–24)	17 (17–23)	23 (21–24)
Number of ridges	11 (10–13)	6–7	10
Length of setae *3a*	12 (11–17)	16 (15–21)	14 (10–15)
Separation of setae *3a*	15 (13–17)	18 (14–19)	15 (14–18)
Length of setae *c2*	33 (28–35)	42 (38–54)	30 (25–33)
On annulus no.:	11 (9–12)	12 (12–14)	9 (8–10)
Length of setae *d*	38 (33–57)	63 (58–73)	48(45–60)
On annulus no.:	25 (25–27)	26 (23–29)	24 (21–26)
Length of setae *e*	11 (9–16)	14 (14–18)	10 (8–17)
On annulus no.:	46 (39–46)	41 (37–45)	38 (36–41)
Length of setae *f*	23 (22–30)	37 (31–37)	22 (18–32)
On annulus no.:	60 (56–66)	58 (55–63)	56 (52–60)
Number of dorsal annuli	33 (29–35)	41 (38–44)	35 (30–38)
Number of ventral annuli	66 (64–70)	63 (57–67)	61 (57–65)
Length of setae *h1*	2 (2–3)	4 (3–4)	4 (3–4)
Length of setae *h2*	49 (24–59)	62 (60–75)	43 (43–55)

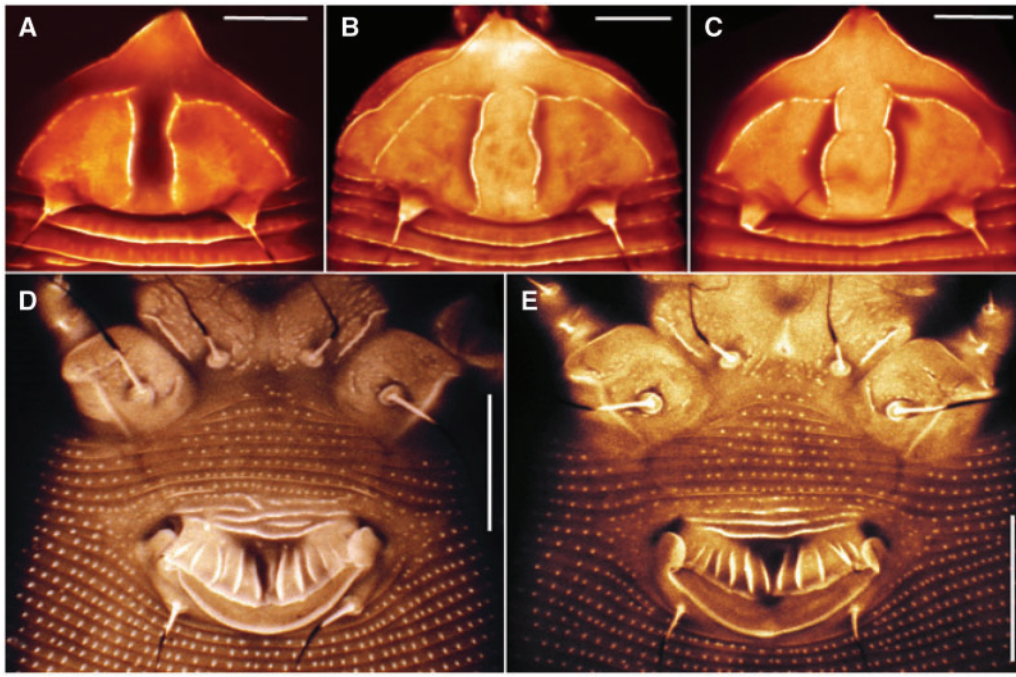

Fig. 5. CLSM images of prodorsal shields (A–C) and coxigenital area (D, E) of *Metaculus diplotaxi* n. sp. from *Diplotaxis tenuifolia* (A, D) and *Metaculus rapistri* from *Isatis tinctoria* (B, C, E). Scale bar = 15 µm.

The mitochondrial marker MT-CO1 used in this study indicated a high level of genetic divergence between the analyzed populations of *M. lepidifolii*, *M. rapistri*, and *M. diplotaxi* n. sp. (ca. 12–22%). The values of divergence between these *Metaculus* populations are comparable to or higher than estimates of interspecific variation in other mite taxonomic groups (Anderson and Morgan 2007, Dabert et al. 2008, Tixier et al. 2008; Martin et al. 2010; Skoracka and Dabert, 2010, Skoracka et al. 2012, Li et al. 2014). These data, in combination with the morphometric results presented above, provide compelling evidence to support the establishment of *M. diplotaxi* n. sp., as a distinct species.

Discussion

Classical taxonomic methods do not always provide sufficient morphological characters to describe a new taxon or to confirm its status, especially in cases involving cryptic species (Skoracka et al. 2002, Navia et al. 2006, Vidović et al. 2010, Skoracka et al. 2012). Indeed, morphological and molecular techniques are frequently combined when determining the taxonomic validity of mite species (Tixier et al. 2010; Glowska et al. 2012, 2013; Lewandowski et al. 2014). The correct identification of species is important not only in basic research, but also if the taxon is the subject of an applied project. For example, reliable species identification is a key element in the process of vetting a potential biological control agent and introducing it to a targeted region (Henry and Wells 2007, Navajas and Navia 2010, Smith et al. 2010).

The results of the presented study of genotypic and phenotypic variability effectively separated three species of the genus *Metaculus* collected from different confamilial host plants. This is a significant result given that *M. lepidifolii* is a candidate biological control agent of *L. latifolium* that to date has never been collected from another host plant (M. Cristofaro, personal communication). The general morphological similarity between the recently discovered *M. diplotaxi* n. sp., and *M. lepidifolii* presented the possibility that the new population may have represented a population of *M. lepidifolii* that was inhabiting *Diplotaxis tenuifolia*. The results of this study refute that hypothesis.

The results of the phenotypic variability and of MT-CO1 analyses of *Metaculus* spp. from this study were compared with the phylogeny of their hosts within the Brassicaceae. The genera *Diplotaxis* and *Isatis* cluster in the same clade (Clade II), while *Lepidium* belongs to a separate clade (Clade I; Al-Shehbaz et al. 2006). These relationships, combined with the morphometric (see Fig. 1) and genetic (see Table 4) data presented in this study, suggest the possibility of coevolution of these species of eriophyoid mites with their host plants.

Finally, in the interests of gaining a more comprehensive understanding of the taxonomic status of *M. rapistri*, the authors feel that it will be necessary to perform similar morphometric and molecular analyses on samples of this species collected from *R. rugosum*. The results of such analyses could be compared to the data presented here for *M. rapistri* collected from *I. tinctoria*, which is also a target of biological control, to determine if *M. rapistri* is indeed able to feed on hosts

in two different plant genera, a relatively uncommon occurrence for Eriophyidae (Skoracka et al. 2010). If the *Metaculus* populations from these two hosts are revealed to be two distinct species, the mite attacking *I. tinctoria* may also be considered as a candidate biological control agent.

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